Final Report

Project Title: Manila Clam Mortality and Health Evaluation

(Grant Number: NA96FD0194)

Submitted to:

Saltonstall-Kennedy Program
National Marine Fisheries Service

Pacific Shellfish Institute
120 State Ave. N.E. #142
Olympia, WA 98501-0600

November 15, 2001

TABLE OF CONTENTS

I. PURPOSE

A. Problem and Opportunity Addressed by This Project

Manila clam industry on the Pacific Coast of the United States. Over 3000 tons of manila clams (*Tapes philippinarum*), valued at over $22 million, were produced on the west coast of the U.S. in 1996 (PCSGA 1998). Most of this production occurs in Washington State although these clams are also produced in California, Oregon, and in British Columbia, Canada. An unfilled domestic and overseas demand is pushing attempts to increase production of this clam. In addition, a significant manila clam seed production industry has developed with production facilities in Washington, Oregon, California and Hawaii. Limited numbers of native littleneck clams (*Protothaca staminea*) are also produced in Washington and Alaska but production is limited due to its shorter shelf life, lower price (for the producer), and consumer preference for the manila clam. According to Johnson (1996), hatchery production of manila clam seed should fuel further growth in this industry.

This industry has enormous potential for growth both in terms of market ready product and also as seed product, for which there is a large demand worldwide, as long as the seed meets minimum health requirements. If it can be increased, the high value Manila clam production can replace demand for imported clams (almost 8000 tons in 1996), provide wholesome product to domestic consumers, and help alleviate the U.S. trade imbalance through exports of product. Asian demand for west coast shellfish products has grown significantly in the last five years.

Littleneck and cherrystone clams (i.e. the hard clam*, Mercenaria mercenaria*) are also extremely popular on the east coast of the U.S., and production has not met demand. An infectious disease of hard clams (QPX) was recently reported from the Canadian Maritime Provinces and from Massachusetts (Whyte et al. 1994; Smolowitz et al 1998). This disease caused mortalities primarily in one and one-half to two year-old clams. Fortunately, no similar disease has been reported in manila clams from the west coast of the United States. However, investigations have been very limited and, as a result, there has been no effort to develop a management strategy for any diseases that might occur in the future. In addition to the need to develop such health data, information gained from a health evaluation of manila clams on the U.S. west coast will also be of value in future evaluations of eastern hard clams.

One of the most serious constraints to industry growth on the west coast of the United States, according to clam producers, is the occurrence of sporadic mortality and poor growth, due to unknown causes. Mortalities have been reported in fall and winter months from November to March with some exceptions. Freezing damage may be a factor in some clam mortalities. Freezing damage was believed to be the cause of manila clam mortalities in British Columbia in 1989 (Bower 1992).

No highly pathogenic infectious diseases of manila clams are known to occur on the west coast of North America. Although some studies have been conducted in Canada (Bower 1992) there has been limited evaluation of the problem, particularly in the U.S. The manila clam is also widely cultured in Europe where it is extremely desirable, but production has been limited by a bacterial infection known as brown ring disease (BRD) (Paillard 1995). While BRD has not been reported from the U.S. west coast, it is important to exclude BRD and other pathogens, which could become established, particularly if a health management program is not in place.

In order to resolve the problem of clam mortalities and formulate solutions, allowing for further growth of the domestic clam industry, the following needs were identified at a Sea Grant Shellfish Growers Conference (Olympia, Washington, March 1998):

- Growers agreed that clam mortalities and lost growth are a serious problem but recommended that more quantitative and systematic data describing the extent and severity of the problem and the role of environmental factors be developed.
- Growers were concerned that there was extremely limited information available regarding clam health and diseases in Washington, Oregon and California and recommended that such information be systematically collected.
- Some mortality in winter appears to be related to freezing temperatures and excessive freshwater inflows, but there is insufficient knowledge to confirm this diagnosis in specific cases. A study to define the environmental limits and diagnostic features of freshwater morbidity and mortality and freezing effects was recommended.

In addition to fulfilling these needs, the study supported the continued export of manila clams under new requirements set by the World Trade Organization (WTO) rules to which the United States is a signatory. The recent formation of the WTO was followed in 1994 by the recognition of the Office International des Epizooties (OIE) as the arbiter of non-tariff trade disputes. The OIE has developed a shellfish disease list which is important to U.S. shellfish producers because now all shellfish placed in receiving waters are subject to the requirements for disease free certification and disease free production requirements developed by OIE. Such disease free certification must be based on studies such as the health survey conducted in this study. To our knowledge, there has never been a systematic survey of manila clam health and disease on the west coast of the United States.

B. Objectives of the Project

The overall goal of the project was to initiate the establishment of production standards and a health baseline for intensive clam production on the west coast of the United States. The baseline data on manila clam health can be used to assist state and tribal shellfish biologists in assessments of public and tribal clam resources. The following tasks were proposed in order to implement these objectives and were fulfilled, with small modifications, necessitated by results obtained during the study and additional input from shellfish producers during the study.

Task 1. Cooperative industry clam survival and performance study. Sites of intensive clam production were monitored over a one year period using defined plots in which clam growth, survival, condition and health was measured in conjunction with monitoring for a variety of environmental parameters. Clam growers participated directly in the study.

Task 2. Survey of clam health and disease in Washington, Oregon and California. Adult and seed clams were examined histologically for the presence of infectious diseases to initiate a health baseline for clam production areas. As a result, a baseline of clam health for the west coast was established.

Task 3. Evaluation of short-term freezing and freshwater exposure effects on clams. Experimental studies were conducted at a clam production facility to define, in detail, tolerance of various clam populations to low salinity exposures, followed by recovery periods. Additionally clams were analyzed by detailed necropsy and histology methods in order to define the pathological criteria for the diagnosis of low-salinity and freezing condition exposures.

Task 4. Clam mortality response team. The project staff, along with associated scientists from the University of Washington responded to several clam mortality events during the study.

II. APPROACH

A. Detailed description of the work performed

Task 1: Cooperative PSI - industry clam survival and performance study.

Four sites at two locations were selected and monitored over a 19-month period (late October 1999 to May 2001). These sites were located on the commercial clam growing properties of the Little Skookum Shellfish Growers at Little Skookum Inlet and Chelsea Farms on Eld Inlet in south Puget Sound (Figure 1).

The sites varied in terms of water circulation and substrate composition. Little Skookum Inlet is a narrow and shallow extension of Totten Inlet. It has significant tidal exchange and much of the water in the inlet drains at each tide cycle. Eld Inlet, on the other hand, is a wide and relatively deep water-body. Tidal circulation is moderate but strong wave and wind forces can occur. One of the two sites in Little Skookum Inlet (designated "Plant") was adjacent to a small perennial stream (Lynch Creek) and was dominated by gravel-shell material, while the other site ("Slough") was mainly in fine-grained sediment near the head of the inlet. Both of the Little Skookum sites were known to be influenced by sheet-flow and stream-fed freshwater runoff during heavy rainfall events. Clam mortalities have been previously observed at the Slough site. Fine to medium sand was the dominant sediment type at both of the Eld Inlet sites ("Creek" and "Beach"). The Creek site had sheet-flow conditions during heavy rainfall, and near year-round groundwater runoff. There was little or no groundwater runoff at the Beach site.

Two clam sizes were tested in this study, small seed and one-year-old juveniles. Seed measuring 6 to 10 mm shell length was obtained from Kuiper Mariculture; Bayside, CA and 1-year-old juvenile clams were obtained from Chelsea Farms; Olympia, WA. The juvenile clams also originated from seed grown at Kuiper Mariculture. Representative shell lengths and wet weights were collected for each age class.

Each treatment consisted of eighteen "Vexar" sample bags (approx. 2 ft^2 and 6mm-mesh) stocked with an industry standard density of 450 each of either yearling or seed clams. These bags were placed at an elevation of +3 to +4 feet (MLLW) for each site. A total of 144 bags were deployed. Bags were partially embedded in the substrate and held in place with iron rebar "staples."

- For each age class, a total of 12 bags were examined for each sample period (2 sites, 2 plots per site, and 3 bags per plot) every five to seven months. Additional bag capacity was available to allow sampling during unusual rainfall or temperature events.
- Mortality was evaluated by examining all clams within each sample bag and tabulating the results.
- Mean length was estimated by measuring the shell lengths of 50 to 100 clams from each sample.
- Growth rates were determined by comparing length and weight over time within treatments.
- Health and condition of tissues was evaluated by examining a total of 150 clams histologically at each of two of the sampling locations.
- Salinity, and water and sediment temperature data were collected at each experimental site using automated monitors. These data were logged every five minutes during the winter 1999 to 2000 period. In addition, the Little Skookum Shellfish Growers installed a recording salinometer in late 2000 at a deep-water site to gather a continuous temperature and salinity record.

Task 2: Survey of clam health and disease in Washington, Oregon and California.

Adult and seed clams were examined histologically for the presence of infectious diseases over an eighteen-month period to initiate a health baseline for clam production areas. Where available at least 150 clams were examined from each location to meet O.I.E. standards. Histological evaluation was performed on clams fixed in Davidson's fixative and prepared by conventional paraffin embedding methods used for shellfish (Elston et al. 1987). Since brood stock maintenance takes place exclusively in Washington, all brood stock clams examined were from Washington.

In addition, clam seed from seed rafts or intensive production facilities in California, Oregon and Washington were examined. In order to meet clam grower requests for examinations of seed, within the limitations of the project, some seed samples were examined with a sample size of 60, rather than 150 seed per sample.

In the proposal, we committed to the examination of a total of 1110 clams by histological methods. We actually examined a total of 1184 clams during the study, although some modification of sample location, date and number of samples was required by availability of clams and grower requests for specific examinations.

Task 3. Evaluation of short-term freezing and freshwater exposure effects on clams.

We conducted detailed experimental studies at a clam production facility in Quilcene, Washington. We proposed to conduct detailed experiments to define freezing and freshwater exposures, followed by recovery periods. After initial experiments on freezing damage confirmed the findings of other investigators, as cited in the proposal (Bower 1992), it seemed prudent to focus our primary effort on freshwater and low salinity effects on manila clams. We also received further input from shellfish growers during the study requesting as much

information as possible on low salinity exposure effects on clams. In addition, our early experiments during this study indicated that the response of manila clams to low salinity was more complex than originally anticipated, depending on the clam source and on a variable ability of individual clams to maintain a survival or shell closure response. As a comparative assessment of the proposed scope of work and the slightly modified scope of work, we originally proposed 20 experimental exposures of 240 clams total. The actual work completed took place over 16 month periods and included over 30 exposures of more than 4500 clams.

Task 4. Clam mortality response team.

The project staff responded to three clam mortality events reported during the study. The response consisted of environmental and pathological characterization of affected clams.

B. Project Management and Organization

Dr. Ralph Elston, Ph.D., Pacific Shellfish Institute, was the technical project manager, responsible for project organization and implementation, specifically including pathological characterization and experimental exposures. Dr. Daniel Cheney, Ph.D., Executive Director of the Pacific Shellfish Institute, was responsible for field and environmental components of the project and also managed the administration and financial control of the project. Mr. Brian MacDonald and Mr. Andrew Suhrbier of the Pacific Shellfish Institute provided technical support for the project.

III. FINDINGS

A. Actual Accomplishments and Findings

Task 1: Cooperative PSI - industry clam survival and performance study.

We observed wide variations in the survival, and growth of clams at the experimental sites. There was very low mortality and few empty shells were observed throughout the study period at the Chelsea Farm site. Natural recruitment into the sample bags resulted in a modest increase of clam survivors at the end of the experiment in three of the four treatment groups (Figure 2, top). These naturally recruited clams accounted for up to 10% of those sampled from each growout bag at the conclusion of the experiment. There appeared to be little or no recruitment to the seed bags at the Creek site.

Yearling clams at Little Skookum Slough site also showed a modest gain in numbers during the first 12 months, but seed clam numbers declined precipitously during this same period. Yearling clams at the Slough site had high losses between the 12 and 19 month sampling, but additional seed naturally recruited into the growout bags during the spring 2001 season (Figure 2, bottom). Clam survival was stable at the Little Skookum Plant site in the first 5 months. Unfortunately, the grower inadvertently over planted a large number of seed clams at the Plant plot during the summer 2000 period. These seed clams were indistinguishable from the seed and yearling clams in the growout bags during our fall 2000 survey and resulted in elevated counts in the sample bags. Survival of the original and newly seeded groups was poor at the Plant site between 12

and 19 months, and was comparable to survival of yearlings at the Slough site (Figure 2, bottom).

Length and weight data were obtained from all treatment groups at the initial planting, and at 5 (lengths only), 12 and 19 months post planting. Detailed analyses were only made on the Chelsea Farms data. Average length and weight data for Manila clams at the Chelsea Farms are shown in Figure 3 for the "Beach" and "Creek" stations. There were significant differences in total length between 0 and 5 months and 5 and 12 months post-plant (99% confidence, one-way ANOVA test) in the seed and yearling animals, and growth during the first year of the experiment was excellent. Growth as measured by length of both groups was insignificant between 12 and 19 months post plant (Figure 3, top). Seed clams, however, gained only a slight amount of weight during the first 12 months. Yearlings, which began at or near the average weight of seed clams, more than doubled in average weight. There were modest weight gains in both seed and yearling animals between 12 and 19 months (Figure 3 bottom).

Air temperatures, and water column and sediment pore water temperatures were nominal during the winter 1999 and 2000 seasons. Winter 1999 data plotted in Figure 4 indicate short-term periods of low air temperatures as low as -2 ^{o} C. These usually corresponded to the nighttime low tides. While freezing damage had been reported on clam grounds in Puget Sound prior to 1999, water column and sediment pore water temperatures were well above freezing throughout the winter 1999 and 2000 periods.

Rainfall and salinity data for the Chelsea and Little Skookum sites are shown in Figure 5. Total rainfall during the winter 1999 to 2001 period was below normal and the salinity remained relatively constant at all four sites. There was a sharp decline in salinity at the Little Skookum Slough site; however, we believe the measurements to be a result of instrument error.

There were brief periods of reduced salinity corresponding to short-term rainfall events. Sharp reductions in salinity to 10 psu and below were likely the result of a thin layer of freshwater overlying more saline water. This freshwater layer did not appear to have a measurable affect on water column salinity at the study sites. In general, it appears that the water conditions observed during the winter 1999 and 2000 periods were within the normal ambient range of Manila clams.

Task 2: Survey of clam health and disease in Washington, Oregon and California.

Tables 1 and 2 show results of the health survey for clams sampled during the project. The proposal committed to the examination of 1110 clams from scheduled survey collections, not including those examined for mortality response or experimental tasks, and a total of 1184 clams were actually examined. The locations of sampling were adjusted slightly from the original proposal upon consultation and request from industry members. Brood stock clams were not available from Oregon or California but seed clams were available from Washington, Oregon and California.

<u>Clinical and histological description of lesions:</u>

Histological findings for adult clams are summarized in Table 1 and results for seed clams are shown in Table 2. Several conditions relevant to the health of clams were identified but no diseases considered certifiable by the O.I.E. or West Coast state governments were found in this study.

Mytilicola. *Mytilicola orientalis* is a metazoan parasite that occasionally infests the digestive tract of manila clams, as well as Pacific oysters. It has been known to occur in Pacific oysters in West Coast culture sites from California to Washington since the 1930s (Wilson 1938), but it typically occurs at a relatively low prevalence.

Recognized infectious diseases of the manila clam. Tables 1 and 2 show that none of the infectious diseases considered reportable by the O.I.E. were found during these examinations.

Resolving parasitic lesion – gonoduct. This lesion was well encapsulated and infiltrated with host hemocytes, indicating that it was resolving. The parasite was degenerated and could not be identified. However, fragments of possible carapace were observed, indicating an arthropod identity. The parasite could be *Mytilicola*, although this species has not been previously reported in the gonoduct of the manila clam.

Digestive gland condition. Digestive gland epithelial height is an indicator of recent feeding activity. Variable digestive gland height in seed populations may indicate lack of adequate size grading and insufficient food supply. A low digestive gland height is indicative of inadequate food supply and is typical of winter conditions when seed have been planted to field sites.

Rickettisia-like prokaryote. Rickettisia-like prokaryotes (RLPs) have been observed in manila clams previously (Elston et al. 1985). Based on histological assessment, the occurrence of these intracellular bacteria appear to be benign and, at the intensity observed in this and other studies, of no pathological significance. Recent work in California (Friedman et al. 2000) has shown that RLPs are a causal factor in abalone mortalities. To ensure that the manila clam RLP is not related to that causing abalone mortality losses, a sample of the manila clam organism was provided to Dr. Friedman's laboratory and tested by an *in situ* molecular probe for identity with the abalone organism. This test confirmed that there is no relationship between the manila clam and abalone RLP.

Task 3. Evaluation of short-term freezing and freshwater exposure effects on clams.

Freezing damage assessment. Freezing damage to manila clam tissue was evaluated by subjecting groups of clams to freezing temperatures (-20°C). Tissue damage associated with cell crystallization and rupture was noted, similar to that reported previously by Bower (1992).

Our initial hypothesis was that clams exposed to low salinity exposures might be more susceptible to freezing damage than clams in high salinity seawater. However, our initial tests with low salinity exposures indicated that the low salinity response occurred in the 10 to 15-psu range, a higher salinity than we would expect a significant alteration in freezing temperature (in comparison with freshwater). Due to the complexity observed in response to low salinity exposures, and based on consultation with shellfish growers, we determined that a greater focus on the proposed low salinity exposures, rather than on freezing damage, was merited. As a result, we significantly expanded the experimental investigation of low salinity exposure.

Low salinity exposure assessment. Experimental evaluation of low salinity effects was made in two systems we designed and built for this purpose and operated at a commercial hatchery facility in Quilcene, Washington. This apparatus was developed to create a flow-through system capable of holding large numbers of test animals at constant levels of reduced salinities (up to four treatments simultaneously) for extended periods of time (typically ~ one month). This

system was later modified to allow for multiple temperature treatments across a single salinity at a time.

In the initial configuration of this system, sand-filtered seawater and unchlorinated fresh water were pumped into separate headtanks (~200 liters) whose levels were kept constant by standpipes and float valves. A coiled length of vinyl tubing was used as a heat exchanger for the fresh water line to help equalize the temperature of the two water sources. Each of these two tanks fed a manifold fitted with four outlets restricted by variously sized orifices that flowed into mixing tanks. Each mixing tank (~20L) flowed in turn into a treatment tank (~40L) where the test animals were held. Altering the sizes of each orifice feeding into the mixing tanks thereby controlled the salinity of the water within each treatment tank. A continuous flow of mixed algae was introduced into the saline head tank at a rate sufficient to provide excess in all treatments. Air stones were used in each mixing tank to ensure adequate mixing of the two water sources and to maintain DO saturation prior to the water being allowed to enter the treatment tanks. Salinity loggers and periodic manual checks were used to track treatment levels.

Our working hypothesis, with regard to the low salinity effects on manila clams, was that resistance to low salinity consisted of two features, a physiological capacity of the tissues to tolerate a particular low salinity and a survival response, consisting of the time for which the clam can maintain a closed shell condition, thus excluding lethal low salinities. Clearly the survival response is complex, and depends both on aspects of the clam's metabolism (e.g. capacity for anaerobic metabolism) and possibly on environmental factors, such as temperature. Initially, to separate the physiological response from the survival response, we conducted several experiments in which we removed a portion of the clam's shell (Figure 6), so that we effectively removed the survival response and could observe the isolated physiological response (capacity of the tissues to function at particular salinities. This approach was very useful in helping to establish the lower physiological limits of salinity tolerance. After these initial experiments, we proceeded to confirm the physiological response in intact clams through long-term exposure, in which the clams would eventually have to open their shells and physiological tolerance could be determined. In addition, survival response could be measured in such experiments by measuring the length of time required before a mortality response was manifested at what we had previously established as lethal low salinities. The series of experiments conducted during this study to evaluate low salinity tolerance is shown in Table 3.

Results of experiments 1 and 2:

The purpose of experiments 1 and 2 was to develop and test the shell cutting method as a means to assess physiological tolerance to low salinities and to obtain data regarding physiological tolerance. In experiment 1, the shell cut control clams had a mortality rate of between 50% and 60% mortality. The slowly increasing mortality in all treatments and cut control demonstrated that the shell cutting method needed to be improved and that we needed to define the duration of the recovery period. Experiment 1 did show that a three-day exposure to very low salinities (less than 10 psu) is sufficient to produce a mortality effect in clams exposed directly to low salinity water as a result of having a portion of the shell removed. The shell cutting method was improved in the second experiment but mortality still reached 50% in some groups due to the

shell cutting procedure. We were able to conclude from the experiments that all groups of clams are intolerant of salinity below 10 psu.

Results of experiment 3:

The rationale of this experiment was to determine the response of clams over a range of short-term exposures to various salinities. The results showed that there was no mortality that could be accounted for by low salinity in any 2 or 4-day flow exposures, even after a 21-day recovery period. This experiment showed that clams could effectively exclude, by their shell closure survival response, a lethal low salinity of 8 ppt for at least four days.

Results of experiment 4:

The purpose of experiment 4 was to confirm the earlier result that some groups of clams showed possible tolerance of salinities as low as 10 psu and to more definitively investigate such possible differences in low salinity tolerance. The results showed that mortality was less than 60% in all treatments at 10 days post treatment. Results were inconsistent between replicates in some cases but clams from Thorndyke Bay appeared to have a greater tolerance for low salinities than clams from a South Puget Sound site located near a natural freshwater input. We concluded that the variable results could be a result of very high variability of salinity tolerance in the clam populations.

Results of experiment 5:

The rationale for this experiment was to determine the typical tolerance time of clams to low salinities that previous experiments indicated were lethal (i.e. 5 psu and 10 psu). The results, presented in Figures 7 and 8, showed that at 5 psu, a mortality response occurred in all groups exposed for eight days or longer. The response was graded but a dramatic increase in mortality occurred after 10 days of exposure. No mortality was seen in clams exposed for 2, 4 or 6 days. At 10 psu, a mortality response was shown in all groups exposed for 10 days or longer. The response was graded from 10 days to 14 days exposure. No mortality was seen in clams exposed for 2, 4, 6, or 8 days.

We concluded that the clam group tested (Thorndyke Bay clams) could withstand 5 psu salinity exposure for 6 days without losses at 10.5 °C and 10 psu salinity exposure for 8 days without losses at 10.5 °C.

Results of experiments 6, 7 and 8:

The rationale for these experiments was to use intact clams to establish long-term salinity tolerance. The objective was to establish whether or not there were any differences in the salinity tolerance among different environmental sources of clams and to verify the physiological tolerances found using the clams with cut shells.

In this series of experiments, clams were exposed to four salinity concentrations over a four-week period, as well as to ambient salinity (30 psu +/- 2 psu) and mortality was recorded. The salinity test concentrations were initially set at 10, 15, 20 and 25 psu for experiment 6 but adjusted to 10, 12.5 15 and 17.5 psu for experiments 7 and 8. In the three experiments multiple

groups were tested, including repeated testing on several groups (Table 4). Two representative responses to testing are shown in Figures 9 and 10. Figure 9 shows the response of a population of clams in which nearly all individuals were tolerant to 12.5 psu while Figure 10 shows a population in which no clams were tolerant to 12.5 psu. Other clam populations contained a variable number of individuals with tolerance to 12.5 psu.

From these experiments, we concluded that 12.5 psu is the marginal salinity for manila clams. Most populations appear to contain at least a few individuals with tolerance for 12.5 psu and in exceptional populations, most or all individuals are tolerant of 12.5 psu. No clams were physiologically tolerant to 10 psu and most clams tested were physiologically tolerant to 15 psu, although a few populations showed some loss at 15 psu.

Results of experiment 9:

The rationale for this experiment was similar to that for experiments 6 to 8 but, using the information gained from these previous experiments, we tested four selected stocks of clams, including a brood stock group reported to be the survivors of a seed group suffering high mortality after being placed in a low salinity rearing environment. The objective was to document the extent and nature of any differential response of the low salinity brood stock with other, more typical, groups of clams.

The results (Figure 11) showed that the low salinity brood stock did have higher survivorship at the marginal salinity of 12.5 psu compared to the other groups, at least for the first four weeks of treatment but that starting at about four weeks (of the total 4 weeks plus 5 days of treatment) their mortality rate increased until it equaled that of the other groups.

The results shown in Figure 11 are important in that they show that the mechanism of tolerance to marginal salinities in the low salinity brood stock is a survival response (ability to maintain a longer period of shell closure and low salinity exclusion) rather than a physiological tolerance of the tissues to a lower salinity.

Results of experiments 10 and 11:

The purpose of these experiments was to test short-term exposure tolerance of several groups of seed and adult clams to lethal and marginal salinities. This information was needed because it is relevant to establishing the duration of tolerance to such salinities in field planted seed and adult clams.

The results for seed clams are summarized in Figures 12 and 13 and represent the differential response of two populations of clams. At 10 psu (Figure 12), group K (LS) shows that about 40% of the clams succumb to a 7-day exposure and that 100% succumb to a 14-day exposure. In contrast, from population C, all clams survive a 7-day exposure to 10 psu while fewer than 80% succumb to a 14 day exposure. Similar results for the two clam groups exposed to 12.5 psu are shown in Figure 13

Examining the results for two groups of adult clams (Figure 14) shows that at 10 psu about 40% of the clams (group OA) succumb during a 7-day exposure while nearly 100% succumb to a 14-day exposure. However, all clams from group TA survived a 7-day exposure, although nearly 100% succumb to a 14 days exposure. Note that the mortality graphs shown in these figures plot

the mortality during both the exposure and the post-exposure recovery period. Therefore, it can be seen that the mortality response may require more than two weeks to be observable. Fourteen-day exposures to 10 psu, which result in essentially 100% mortality, do not manifest a mortality response at 14 days but at a later time, usually by one week following the 14-day exposure. In addition, these figures show that there are distinct differences in the survival response (duration of time over which lethal low salinities can be tolerated).

Results of experiment 12:

The purpose of this experiment was to make a preliminary investigation of the effects of temperature on salinity tolerance. Three clam sources were tested at the marginal salinity of 12.5 psu at three temperatures (6°C, 12°C and 18°C). The results of this experiment are shown in Figure 15. Mortality rates were relatively low in these clam groups. An elevated mortality was observed in one of three clam groups tested at 6°C, resulting in an averaged mortality rate of the three groups of about 15% at this temperature. While more definitive work is needed, this preliminary result does not suggest that temperature has a significant effect in moderating the low salinity response in clams.

Results of experiment 13:

The purpose of this experiment was to collect additional samples with specific exposure histories for histological analysis. For this experiment, seed clams were exposed to 10 and 12.5 psu for 1, 2, 4, 7, and 14 days. The digestive gland appeared to be the most reliable organ in which to observe changes resulting from the low salinity exposures. Normal clam digestive gland is shown in Figure 16 and the primary features of the histological response to low salinity are shown in Figure 17. The first response is degranulation of digestive gland cells, occurring after about four days of exposure.

Subsequently, at between 4 and 7 days post-exposure, the digestive gland cells exhibit osmotic swelling, accompanied by detachment of some cells from their basement membrane into the lumen of the digestive tubule.

Summary conclusions of low salinity response investigation of manila clams.

1. The physiological tolerance on manila clams ranges between 12.5 psu and 15 psu.

2. Of the seventeen sources of clams tested, all were tolerant to a salinity of 15 psu.

3. There was variable tolerance to 12.5 psu, depending on the source of clams.

4. The variation in tolerance to low salinity appears to be a function of survival response (shell closure related behavior and physiology).

5. With respect to manila clams' ability to withstand short term exposures to lethal low salinity:

- All clams could withstand 6 days exposure to 5 psu.
- Some clams died at 8 and 10 days exposure to 5 psu.
- All clams died at 12 days exposure to 5 psu.
- All clams could withstand 8 days exposure to 10 psu.
- Some clams died at 10 and 12 days exposure at 10 psu.
- All clams died at 14 days exposure to 10 psu.
- The observed mortality response at lethal low salinity may be delayed.

6. There appeared to be little effect of temperature on tolerance time to marginal low salinity.

7. Low salinity causes recognizable tissue damage.

8. Candidates to select for low salinity tolerance are available in most populations but some populations are enriched with more low salinity tolerant clams than other populations.

Task 4. Clam mortality response.

The project staff, along with associated scientists from the University of Washington responded to significant clam mortalities reported during the study. The response consisted of environmental study and pathological characterization of affected clams as follows, appropriate to the individual case. Table 5 lists the responses to clam mortalities during the project.

Results of Investigations:

Case Number 1:

History: Clams were received at the laboratory for examination on November 21, 2000. The clams were collected from Totten Inlet in South Puget Sound, Washington. Clams were observed by Mr. Steve Bloomfield to be very deep in the beach (5 ½ inches to 6 inches to top of shell). Also reported was some loss of pigment around the siphon tip. There was also a suggestion that the shelf life of the harvested clams may be shortened but this was not confirmed. 20 clams measuring 40 mm to 47 mm in shell length were examined at necropsy and by histology.

Results: The live clams all were distinctly blackened in color and had a mild odor of hydrogen sulfide. There were no significant lesions observed in the live clams. The histological findings are shown in the following table. In summary, the clams appeared to be in healthy condition. The digestive glands indicated active feeding and metabolism. The male clams were in a ripe spawning condition but in most of the clams, the reproductive follicles were ruptured. The female clams were in a post-spawning condition. Rickettsia, or intracellular bacteria, were found in the digestive glands of 2/20 (10%) of the clams.

Case 1 summary and interpretation of results. The condition of the digestive glands, along with the condition of other organs indicated that the clams were in a healthy condition. The black coloration of the clam shells is the result of deposition of hydrogen sulfide (H_2S) by H_2S bacteria that tend to be predominant in anaerobic sediments. There were no indications of

compromised clam health that were associated with the black discoloration of the shells. The black coloration is a result of the deep location of the clams. The reason for the deep residence location of the clams is not known.

It is plausible that shelf life could be shortened as a result of associated H_2S bacteria. However, rinsing the clams in clean seawater should reduce this risk. The reason for the asynchrony in the reproductive development of males in comparison to female clams is not known. This is a relatively common occurrence.

Rickettsia-like prokaryotes have been previously reported in Manila clam digestive glands. Usually, the intensity of infection is low and they are not considered to be of significance to the health of the clam. In other bivalves, the infections have been reported to increase in intensity, in some instances, and to be the cause of significant morbidity and mortality.

Case Number 2:

History: Death loss of clams, *Tapes philippinarum*, was noticed in December 2000 from a South Puget Sound site. One sample was sent to the PSI office where whole clams and/or tissues were fixed. A second sample was taken on December 27, 2000 and sent directly to the Sequim laboratory. The results of this second sample are reported here, but the first group of fixed tissues has been archived. On December 28, two groups of clams were received: an apparently healthy group and a sick group characterized by gaping or weak shell closure. The clams were processed by necropsy and histology procedures. The incidence of conditions is estimated as a proportion of the total numbers of clams.

Necropsy Results: *Group 10A (apparently healthy).* 49 clams received with shell length from 40 mm to 50 mm. One of these clams was gaping. All others had normal shell closure strength. 25 of this group were processed for histological examination.

Group 10B. (sick group). 41 clams received with shell length from 40 mm to 50 mm. 8 were wide gapers, 4 were gapers less widely open and 29 had closed shells but most had weak shell closure strength.

Histology results: *Group 10B:* Of the 12 gaping clams, 100% showed loss of gill epithelium (external covering tissue layer of the gills). At least 75% of these clams had evidence of damage to the digestive tubules and stomach. The tissues showed significant postmortem deterioration. Of the 29 clams with weak to moderate shell closure, there was a minimum incidence of 45% (13.29) of loss of digestive gland cell height, loss of digestive gland epithelial cells, and impacted masses in the stomachs consisting of mucus secreted by the clams, bacterial colonies and unidentified microscopic foreign material. The foreign material appeared to be largely abiotic but did contain some remnants of algal cells and structures that could be degenerated exoskeletons from unidentified marine invertebrates. The foreign material was moderately birefringent.

Group 10A. One gaper was present in this group of 25 clams. The remaining 24 clams had weak to strong shell closure. The gaping clam had significant post mortem deterioration. Of the remaining 24 clams, an estimated 33% had low to medium digestive gland height, loss of digestive gland epithelial cells, and impacted masses in the stomachs consisting of mucus secreted by the clams, bacterial colonies and unidentified microscopic foreign material, like that seen from clams in Group 10B.

Case 2 summary and interpretation of results. The impacted material in the stomachs and reaction of the clam digestive organs is clearly the most significant finding. Apparently the clams have ingested some unidentified material that is highly reactive and causes extensive secretion of mucus, while preventing the normal flow of ingested materials through the digestive system. The stasis of this material in the stomach results in continuing mucus secretion. The mucus appears to become somewhat solidified and, over time, is colonized by discrete and well-defined colonies of bacteria. As a result, the digestive epithelium (lining wall of the digestive organs) becomes damaged and the epithelial cells are lost into the stomach and other organs. In addition, the digestive gland itself shows loss of normal conformation due to the fact that normal ingested material of nutritional value is not being transported into the digestive gland.

This condition would lead to death of the clams and can be assigned as a cause of death with a high degree of confidence. The important question, of course, is the identity of the foreign material ingested by the clams. The type of tissue reaction and the lack of a large number of diatom testes suggest that the cause is not a toxic algae. The foreign material is partially birefringent (which indicates that it has some crystalline structure) and could represent the carapace or other hard body parts of a small marine invertebrate.

There is no previous report of this condition in manila clams or any other bivalves, to our knowledge.

A review of any water quality or plankton data available should be undertaken. If the mortality loss has continued, it would be advisable to get another small sample of clams, along with an assessment of materials present in the water or sediments that could be responsible for this condition.

Case Number 3:

History: A previous death loss of clams, *Tapes philippinarum*, was noticed in December and clams were examined (case report 2). Clams from that sample had impactions of unidentified material in the digestive tract.

This follow up sample consisted of two groups: (1) clams from a high tidal height and (2) clams from a deep bed location. 20 clams from each group were examined by necropsy and histological methods.

Results: *Group 1 (high):* All clams in this group had tight shell closure. Histologically, there were no significant lesions in this group. The condition of the digestive glands was rated as high (optimal) in all individuals.

Group 2 (deep): Of a total of 25 clams in this group, 5 were empty shells, 1 was gaping and 2 were in an advanced stage of decomposition. Lesions were observed in this group associated both with the gills and the digestive gland. There was significant loss of covering cells on the gills in 4/20 clams. In an additional 4/20 clams, the digestive gland showed loss of primary absorptive cells and associated cell death.

Case 3 summary and interpretation of results.

The most important finding appears to be the difference between the two groups of clams examined. While the cause of the lesions could not be determined, the clams from the deep group were in poorer condition. A total of 8/20 (40%) had potentially life threatening lesions although the majority of the lesions observed were considered reversible.

While the exact cause of these lesions could not be determined, the effects on the gills and digestive tract may be a result of bacterial colonies that are able to colonize clams at the deeper locations.

There was no evidence of the foreign material in the digestive tract that was observed from the clams sampled in late December.

B. Significant Problems Resulting in Negative Results

There were no significant problems that had negative results.

C. Description of Need for Further Work

This project significantly advanced the information available regarding manila clam health and condition on the west coast of North America. The results indicate several additional areas of investigation and development that should be undertaken. These include the following:

1. A brood stock selection program to investigate the development of a brood line of clams with enhanced low salinity tolerance.
2. Confirmation and more detailed evaluation of the effects of water temperature on low salinity tolerance of manila clams.
3. Continued evaluation of samples of opportunity that result from future clam mortality events to investigate in more detail the findings such as those reported in field case 2 under Task 4 of this project.

IV. EVALUATION

A. Attainment of Project Goals

1. Were the project goals attained?

The project goals were attained. Measured in terms of the number of clams evaluated for health and condition, the number actually examined (1184) exceeded the number indicated for examination in the proposal (1110).

With regard to freezing and low salinity tolerance effects on clams, the project goals were attained, and in fact exceeded, when measured in terms of the number of experiments proposed and number of clams anticipated for use in those experiments (240) compared to the number of experiments completed and number of clams used in those experiments (4500 clams).

With respect to evaluation of field mortality events, the project goals were attained. Such investigations are by their nature samples of opportunity and all such opportunities provided by shellfish producers were evaluated.

2. Were modifications made to the goals and objectives?

There were no substantive modifications in the project goals and objectives. Minor modifications that were necessitated for various reasons are as follows. The location and timing of samples for health and condition evaluation were modified according to the availability of clams and grower requests for examinations, as noted in the text of the report. However, as also indicated, we ensured that the total number of clams examined exceeded the proposed number to demonstrate that the project effort was at least equivalent to that indicated in the proposal.

In addition, the experimental portion of the project placed more emphasis on low salinity tolerance that on freezing tolerance. This was the result of initial investigations that indicated that freezing response of clams was consistent with that reported by other investigators and indications that the low salinity response of clams was more complex than anticipated, indicating that more detailed evaluation was justified. As noted in the previous section, the effort expended in this portion of the project exceeded that indicated it the proposal.

B. Dissemination of Project Results

The project results were presented orally at the 2001 meeting of the Pacific Coast Shellfish Growers Association in Silverdale, Washington. Interest from shellfish producers was high as indicated by discussions generated by the presentation. In addition, all the clam producers who provided shellfish for analysis will be provided with a copy of the final report for the project. In addition, we have submitted an abstract for presentation of the results at the World Aquaculture Society/National Shellfisheries Association Meeting in San Diego in January 2002 and will present the results there pending availability of travel funds.

The principal author of this report was Ralph E. Elston. Co-authors were Daniel P. Cheney, Brian F. MacDonald and Andrew D. Suhrbier.

Pacific Shellfish Institute
120 State Avenue NE #142
Olympia, Washington 98501 USA
Tel: (360) 754-2741
Fax: (360) 754-2246
Email: **psi@pacshell.org**
Web: www.pacshell.org

REFERENCES CITED

Bower, S. M. 1992. Winter mortalities and histopathology in Japanese littlenecks (*Tapes philippinarum* (A. Adams and Reeve, 1850) in British Columbia due to freezing temperatures. J. Shellfish Research 11(2): 255-263.

Elston, R. A., J. H. Beattie, C. Friedman, R. P. Hedrick, and M. L. Kent. 1987. Pathology and significance of fatal inflammatory bacteremia in the Pacific oyster, *Crassostrea gigas*. J. Fish. Dis. 10: 121-132.

Elston, R. A., M. T. Wilkinson, R. Burge. 1985. Occurrence of branchial Rickettsiales-like infections in two bivalve molluscs, *Tapes japonica* and *Patinopecten yessoensis*. J. Fish Dis. 9:69-71.

Friedman, C. S., K. B. Andree, K. A. Beauchamp, J. D. Moore, T. T. Robbins, J. D. Shields, R.P., Hedrick. 2000. *Candidatus Xenohaliotis californiensis*, a newly described pathogen of abalone, Haliotis spp., along the west coast of North America. International Journal of Systematic and Evolutionary Microbiology 50: 847-855.

Johnson, H. M. 1996. Annual report on the United States seafood industry. H. M Johnson and Associates, Bellevue, WA. 103p.

PCSGA (Pacific Coast Shellfish Growers Association). 1998 West Coast Shellfish Production. Pacific Coast Shellfish Growers Association, Olympia, Washington.

Paillard, C, Maes, P 1995. The brown ring disease in the manila clam*, Ruditapes philippinarum*. I. Ultrastructural alterations of the periostracal lamina. J Invertebr Pathol 65: 91-100.

Smolowitz, R., D. Leavitt and F. Perkins. 1998. Observations on a protistan disease similar to QPX in *Mercenaria mercenaria* (hard clams) from the coast of Massachusetts. J. Invertebr. Pathol. 71: 9-25.

Whyte, S. K., R. J. Cawthorn and S. E. McGladdery. 1994. QPX (quahog parasite X), a pathogen of northern quahog *Mercenaria mercenaria* from the Gulf of St. Lawrence, Canada. Diseases of Aquatic Organisms 19: 129-1136.

TABLES

Table 1. Summary of evaluation of adult and brood stock clams for health and condition.

	Brood stock and adult clam location codes[1]		
	WA-1	***WA-2***	***WA-3***
Date of sample:	11-23-99	4-19-00	4-17-01
Number examined:	159	154	151
Findings[2]			
Presumed *Mytilicola*	0	2 (1.3%)	3 (2.0%)
Resolving parasitic lesion – gonoduct	0	1 (0.6%)	0
O.I.E. Reportable Diseases:			
Haplosporidan plasmodia or spores	0	0	0
Mikrocytos mackini	0	0	0
Perkinsus spp.	0	0	0
Marteiliodes chungmuensis	0	0	0
Bonamia or bonamia-like parasites	0	0	0
Marteilia or marteilia-like parasites	0	0	0
Unidentified protistan parasites	0	0	0
Presumptive viral inclusion bodies	0	0	0

[1]Location codes: WA-1, WA-2 and WA-3 are from South Puget Sound and Hood Canal, Washington. [2]Clinical and histological conditions are discussed in detail in text.

Table 2. Summary of evaluation of seed clams for health and condition

Sample No.	Date	Location Code[1]	Number examined	Predominant findings Codes[2]
1	12-1-99	CA-H	66	Variable digestive gland epithelial height
2	1-5-00	CA-H	60	Low digestive gland epithelial height
3	5-11-00	CA-H	103	None
4	10-2-00	CA-H	160	Some loss of digestive gland cells
5	3-7-00	OR	60	None
6	2-1-00	WA-H	72	None
7	1-24-00	WA-H	63	None
8	2-15-00	WA-H	72	None
9	12-8-00	WA-H	64	Rickettsia-like prokaryote 1/64
	Total number examined:		720	

[1]Location codes: CA-H, Humboldt Bay, California; OR, Netarts, Bay Oregon; WA-H, seed production sites, Hood Canal, Washington. [2]Clinical and histological conditions are discussed in the text.

Table 3. Experiments conducted to evaluate low salinity tolerance in manila clams.

Exp. No.	**Start Date**	**Parameters Tested**	**Groups/numbers of clams tested**
1	Jan 2000	0.4 to 32 psu salinity 3 day static exposures Effect of shell cut	2 clam sources 10 clams each, 6 treatment groups, 120 clams total
2	Feb 2000	5.0 to 30.0 psu salinity 3 day static exposures Effect of shell cut	4 clam sources 10 clams each, 5 salinity treatment groups and two control groups, 280 clams total
3	Feb 2000	5.0 to 30 psu salinity 2 day and 4 day flowing exposure	1 clam source, 25 clams each, 4 treatment groups, 2 exposure periods. 200 clams total
4	Feb 2000	5.0 to 15.0 ppt salinity 3 day static exposure Effect of shell cut	2 clam sources 15 clams each x 3 replicates, 3 treatment salinities and two control groups, 450 clams total

Table 3 (cont.). Experiments conducted to evaluate low salinity tolerance in manila clams.

Exp. No.	Start Date	Parameters Tested	Groups/numbers of clams tested
5	Feb 2000	5.0 and 10.0 psu 2 to 14 day flowing exposure with intact clams.	1 clam source at two salinities, 15 clams each for 7 time periods at 2 day increments, recovery monitored, 210 clams total
6	Apr 2000	10.0 to 25.0 psu 28-day flowing exposure with intact clams.	5 clam sources at four treatment salinities, 15 clams per treatment, 300 clams total
7	Jul 2000	10.0 to 17.5 psu 28-day flowing exposure with intact clams.	11 clam sources at four treatment salinities, 15 clams per treatment, 660 clams total
8	Nov 2000	10.0 to 17.5 psu 28-day flowing exposure with intact clams.	4 clam sources at four treatment salinities, 15 clams per treatment, 240 clams total
9	Nov 2000	10.0 to 17.5 psu 28-day flowing exposure with selected populations of intact clams.	4 clams sources at 4 treatment salinities, 30 clams from three groups, 50 clams from one group, 560 clams total
10	Dec 2000	10.0 and 12.5 psu Adult clams, exposures of 1, 2, 4, 7 and 14 days exposure followed by recovery monitoring, intact clams.	Two adult clam sources at two treatment salinities and five exposure periods. 20 clams per group, 400 clams total
11	Dec 2000	10.0 and 12.5 psu Seed clams, exposures of 1, 2, 4, 7 and 14 days exposure followed by recovery monitoring, intact clams.	Three seed clam sources at two treatment salinities and five exposure periods. 20 clams per group, 600 clams total
12	Feb 2001	12.5 psu salinity exposure at three temperatures for four weeks.	Three clam sources, two or three replicates per source, one salinity and three temperatures 388 clams total
13	Mar 2001	10 and 12.5 psu salinity exposure for 1, 2, 4, 7 and 14 days.	One clam source 100 clams total

Table 4. Summary of results of four-week exposures of various clam groups to different salinity levels (experiments 6 to 8).

Source of clams	Life Stage tested[1]	Minimum Salinity with 100% Survival (psu)	Next lower salinity increment tested (psu)	Remarks
Oakland Bay clams	A	15	10	
Chelsea ground clams	A	15	10	
Chelsea yearling clams	A	15	10	
Little Skookum	A	15	10	
Thorndyke Bay	A	15	10	
Little Skookum Creek	A	15	12.5	Partial loss at 12.5
Little Skookum Slough	A	15	12.5	Partial loss at 12.5
Chelsea Seafarms Creek	A	15	12.5	Partial loss at 12.5
Chelsea Seafarms Beach - N	A	15	12.5	Partial loss at 12.5
Stoney Point - Willapa	A	15	12.5	Partial loss at 12.5
Oakland Bay clams	A	15	12.5	Complete loss at 12.5
Low salinity brood stock	A	15	12.5	Partial loss at 12.5 and 15
Thorndyke Bay	A	12.5	10	Most survived at 12.5
Lummi	A	17.5	15	Partial loss at 12.5 and 15
Seed - 1 California	S	15	12.5	
Seed - 2 Washington	S	15	12.5	
Seed - 3 Washington	S	15	12.5	

[1]Life Stage Codes: A, Adult; S, Seed

Table 5. Incidences of clam mortality investigations.

Case Number	Date	Location	Type of Event
1	11-21-00	South Puget Sound, Washington	Clams very deep in beach, possibly also have shortened shelf life.
2	12-27-00	South Puget Sound, Washington	Field mortality event, clams appeared very thin with weak shell closure
3	2-21-01	South Puget Sound, Washington	Follow up from case number 2 to evaluate condition of clams.

Table 6. Summary of microscopic condition of tissues (Case 1).

	Number and (percent) affected
Number of clams examined	20
Females	10 (50%)
Males	10 (50%)
Digestive gland height – high	19 (95%)
Digestive gland height – medium	1 (5%)
Digestive gland height – low	0
Digestive gland height – very low	0
Rickettsia infections in digestive gland	2 (10%)

Key to digestive gland condition: The height of the digestive gland epithelium in the hatchery seed is rated as (1) **high** (indicating normal active metabolism and ingestion), (2) **medium** (indicating a condition at the lower end of the normal range and an animal at risk from insufficient nutrition), (3) **low (**a pathological condition indicating insufficient nutrition or a toxic dietary effect, but a recoverable condition) and (4**) very low** (a distinctly pathological condition indicating insufficient nutritional intake or a toxic dietary effect, that may be unrecoverable in some cases).

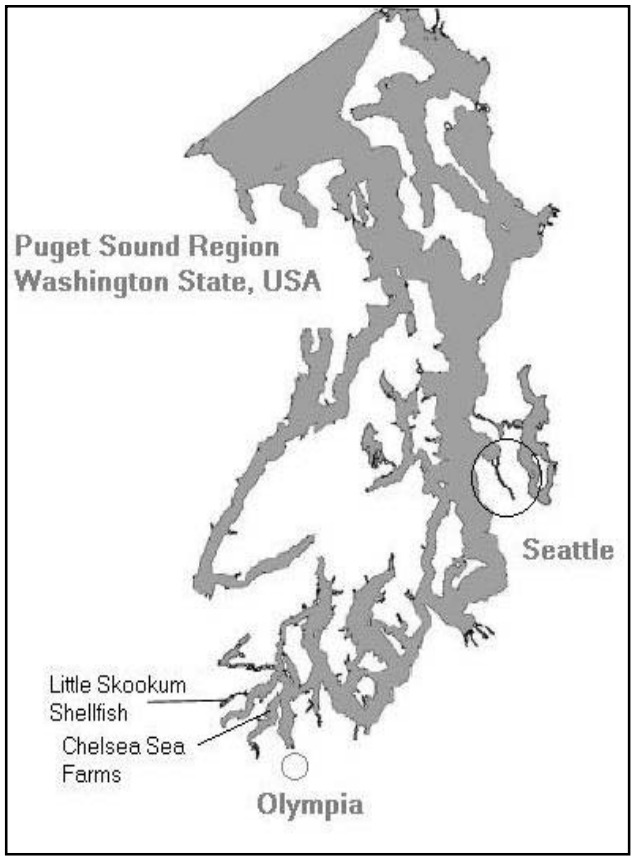


Figure 1. Location of Puget Sound Study Sites

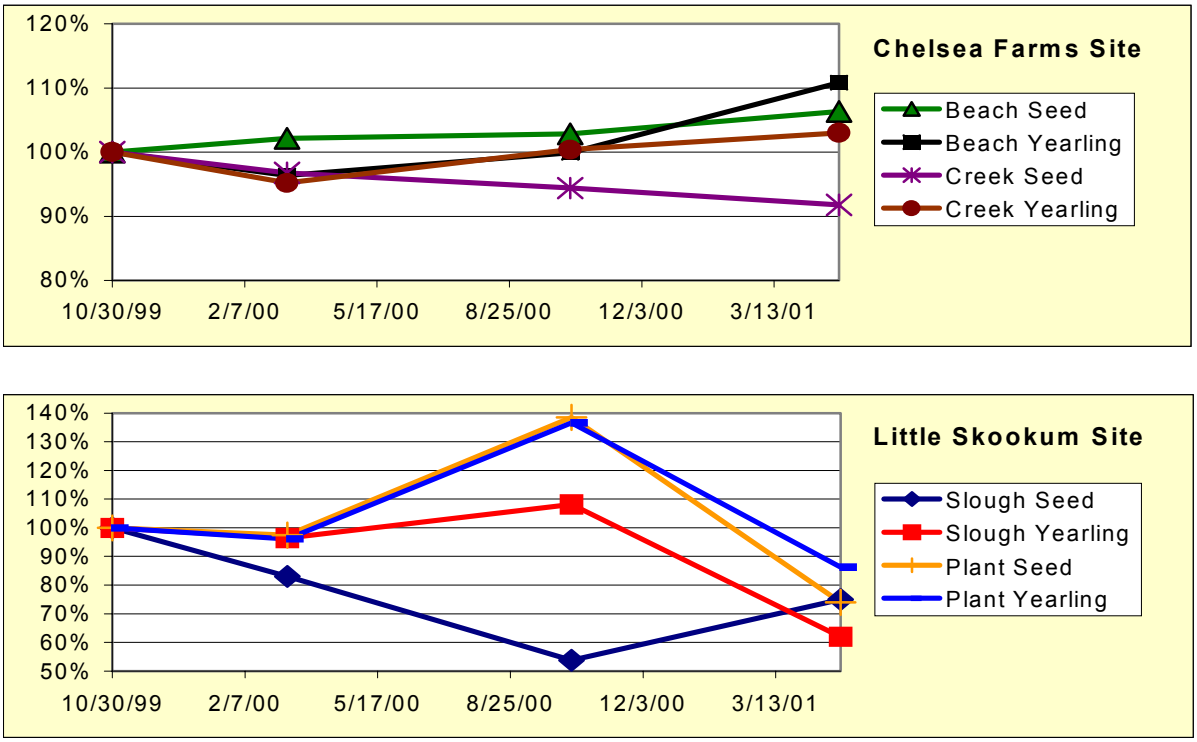
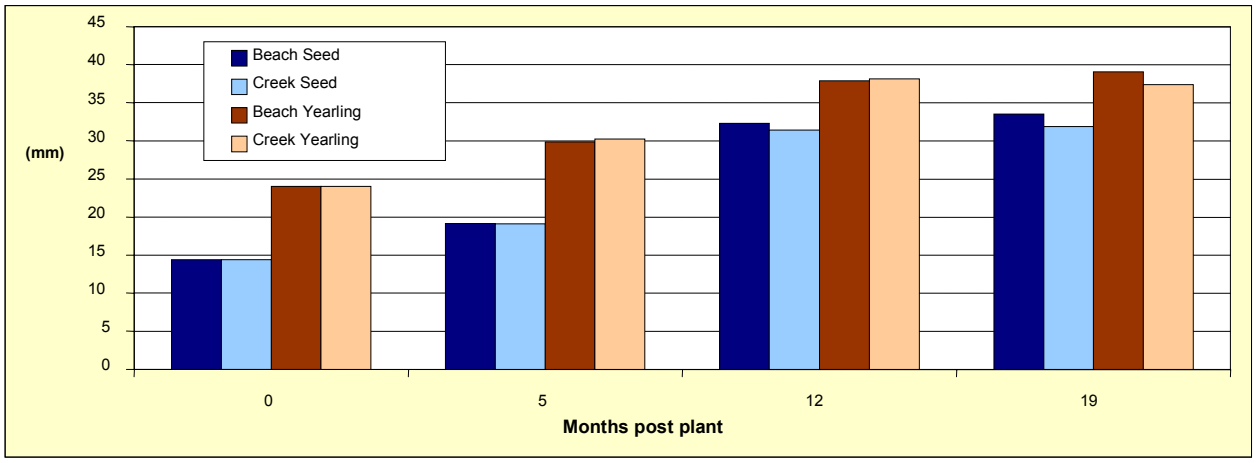


Figure 2. Manila clam survival as percent initial stocking from late October 1999 to May 2001 at Chelsea Farms (top) and Little Skookum Shellfish Growers (bottom).



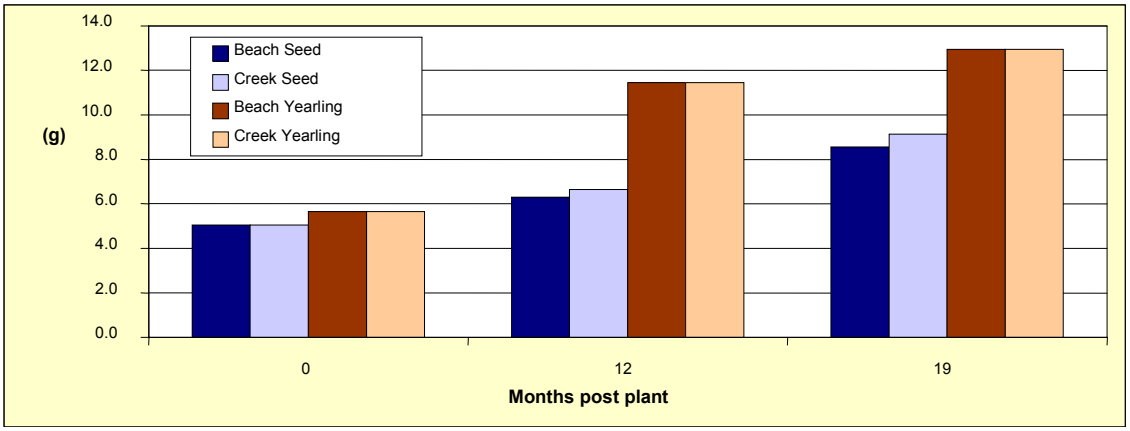


Figure 3. Average lengths (top) and weights (bottom) of seed and yearling clams from experimental plots at Chelsea Sea Farms. Lengths were derived from measurements of 50 to 100 clams in each of 3 replicates per treatment group. Weights are based on the aggregate weights of 50 animals from each replicate.

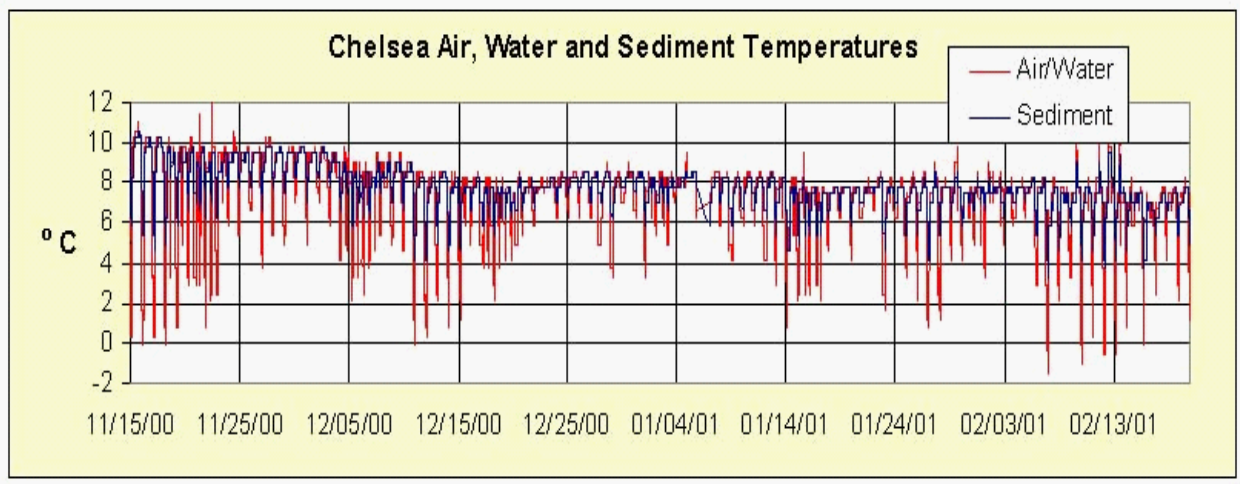


Figure 4. Salinity, water and sediment temperatures at the Chelsea Farms "Beach" site, winter 2000.

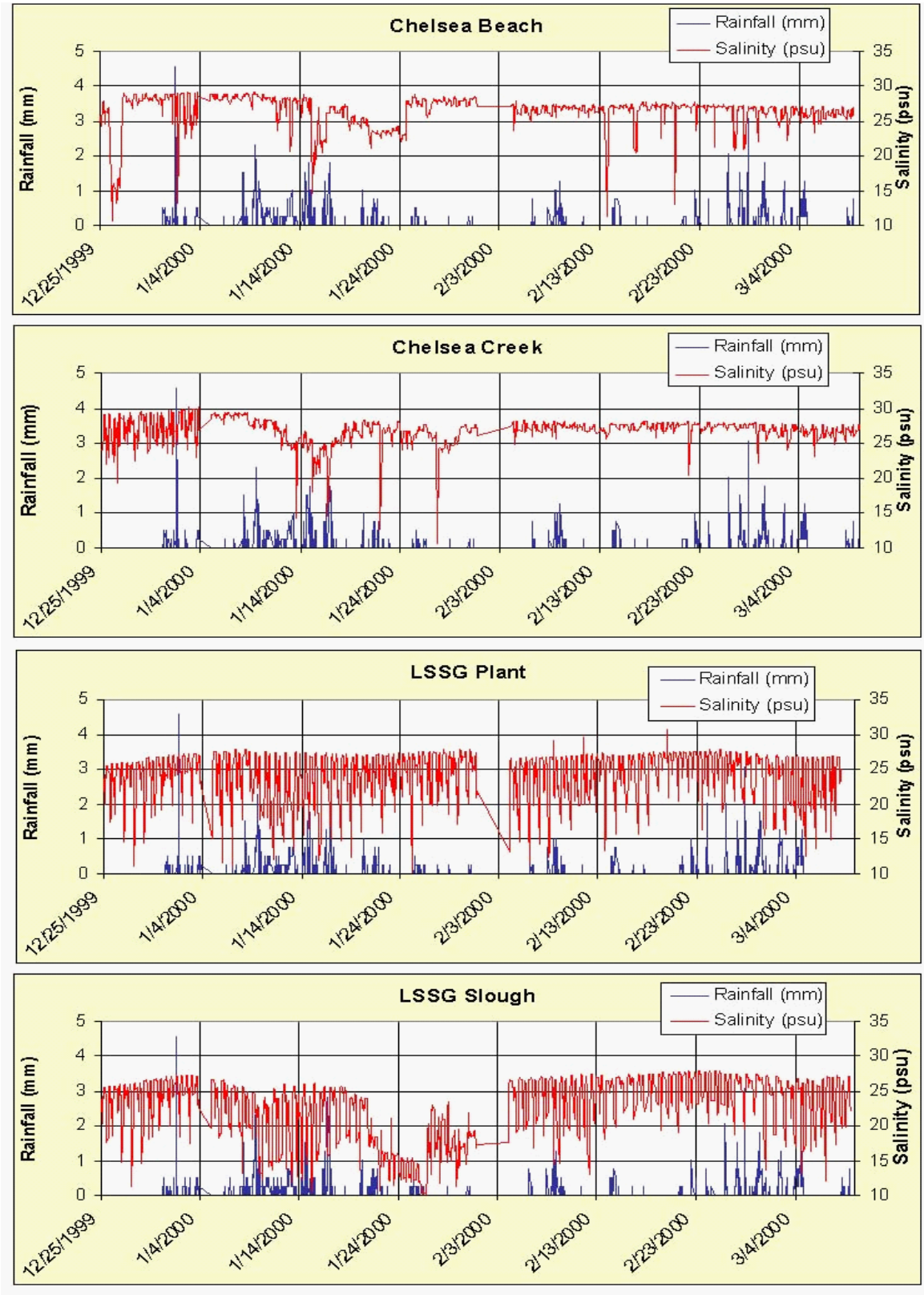


Figure 5. Salinity-rainfall plots at Chelsea and Little Skookum study sites, winter 1999. Rainfall data are plotted in increments of mm per ½ hour.

Figure 6. Manila clam with portion of shell removed to isolate the physiological response to low salinities.

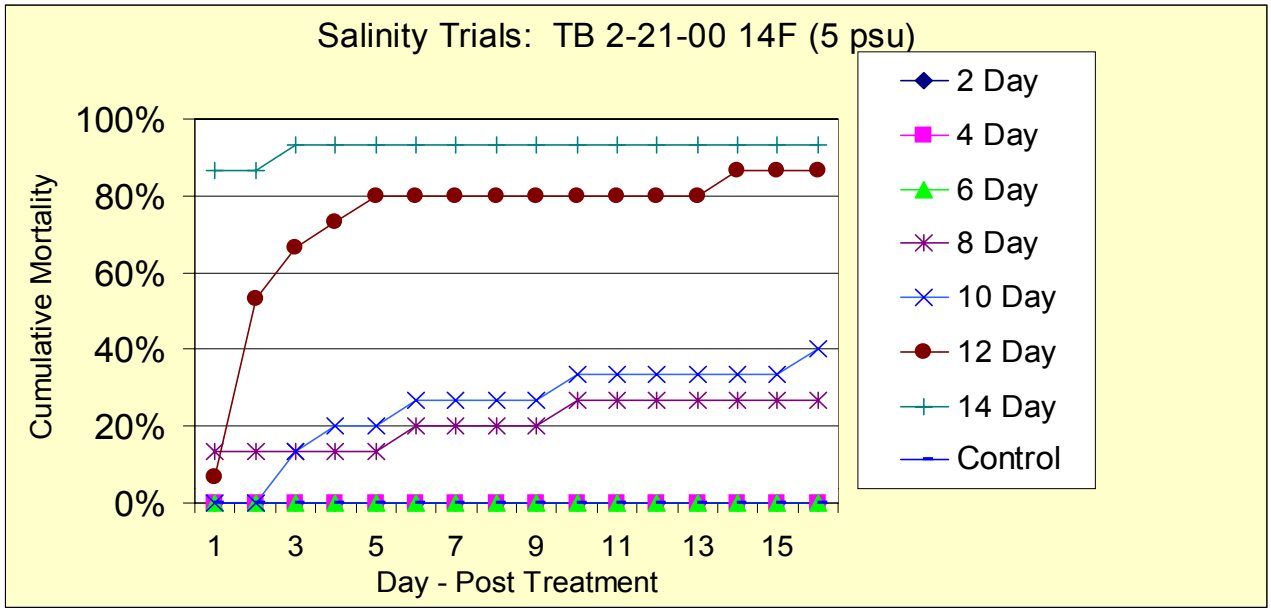


Figure 7. Clam mortality post-exposure at 5 psu. Exposure given at 5 psu for 2 to 14 days.

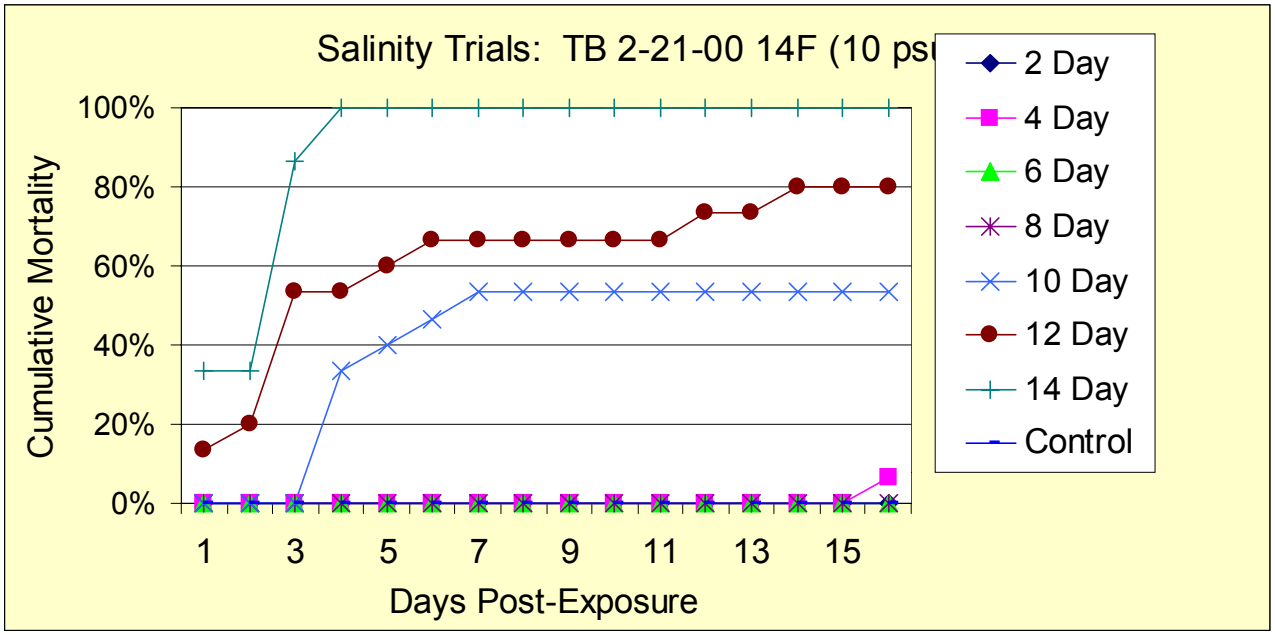


Figure 8. Clam mortality post-exposure at 10 psu. Exposure given at 10 psu for 2 to 14 days.

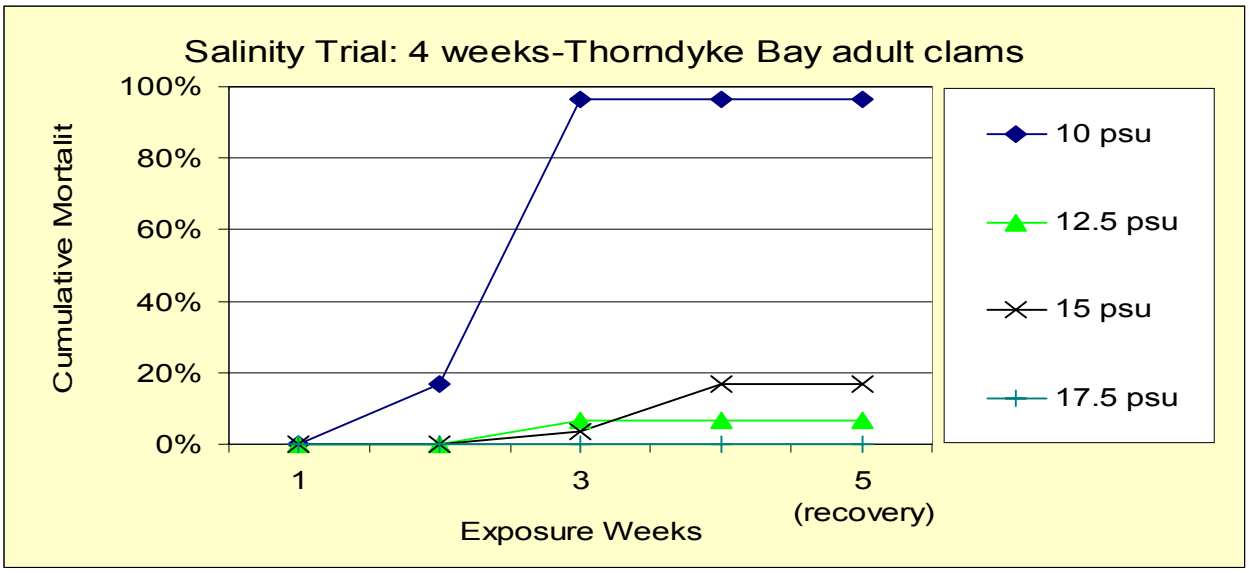


Figure 9. Low salinity tolerant population of clams showing survival of most clams at 10 psu and higher.

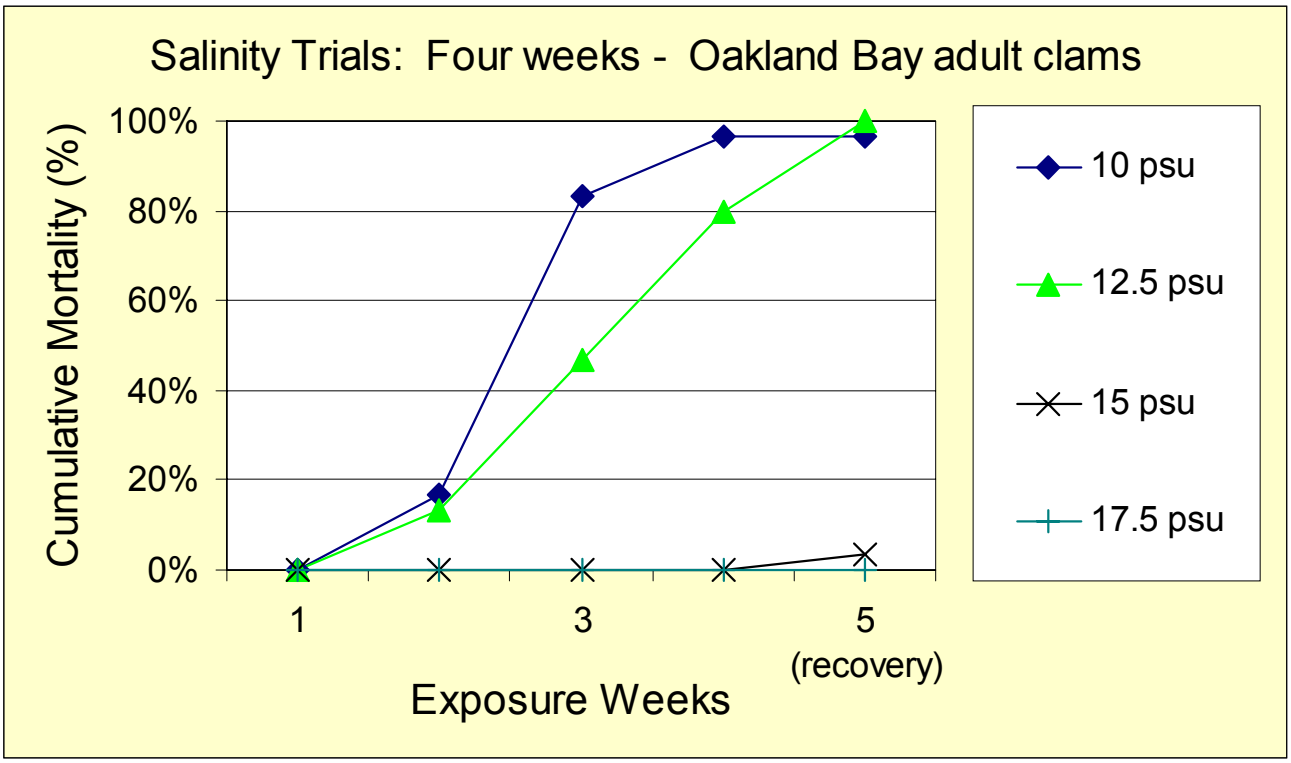


Figure 10. Low salinity sensitive population of clams showing complete loss at both 10 and 12.5 psu but complete tolerance to 15 and 17.5 psu.

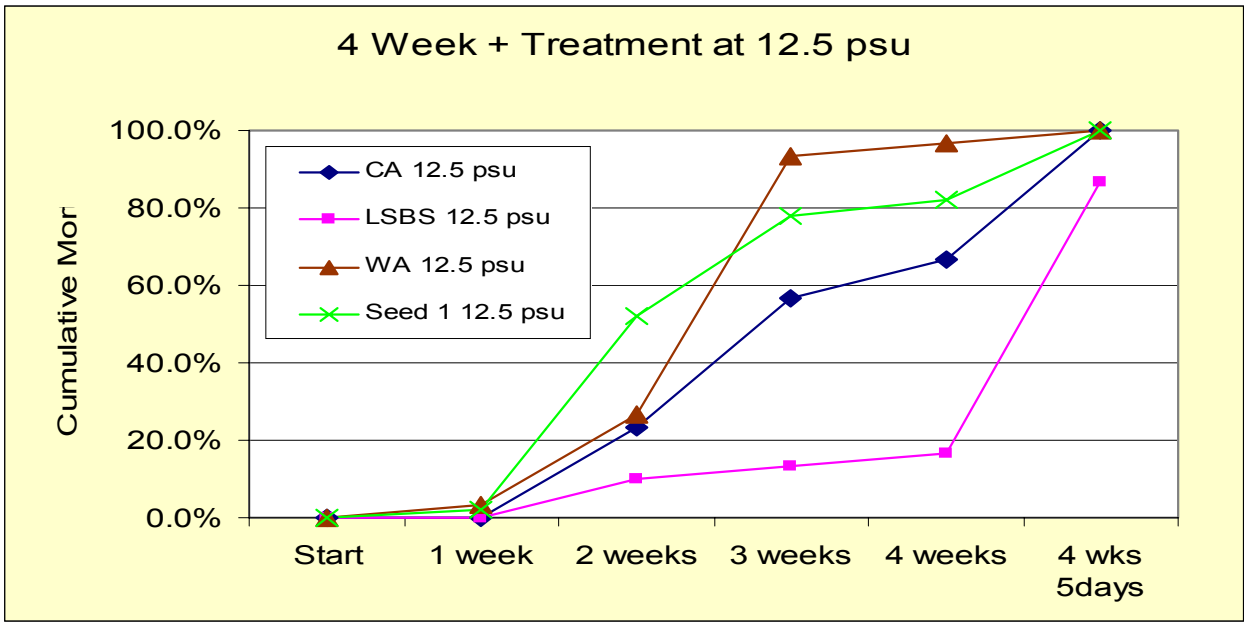


Figure 11. Results of exposure of selected groups of clams to the marginal salinity of 12.5 psu. The populations tested included a California stock of adult clams (CA), a Washington brood stock representing survivors of a low salinity event (LSBS), a population of adult clams from Washington and a population of seed clams produced in Washington.

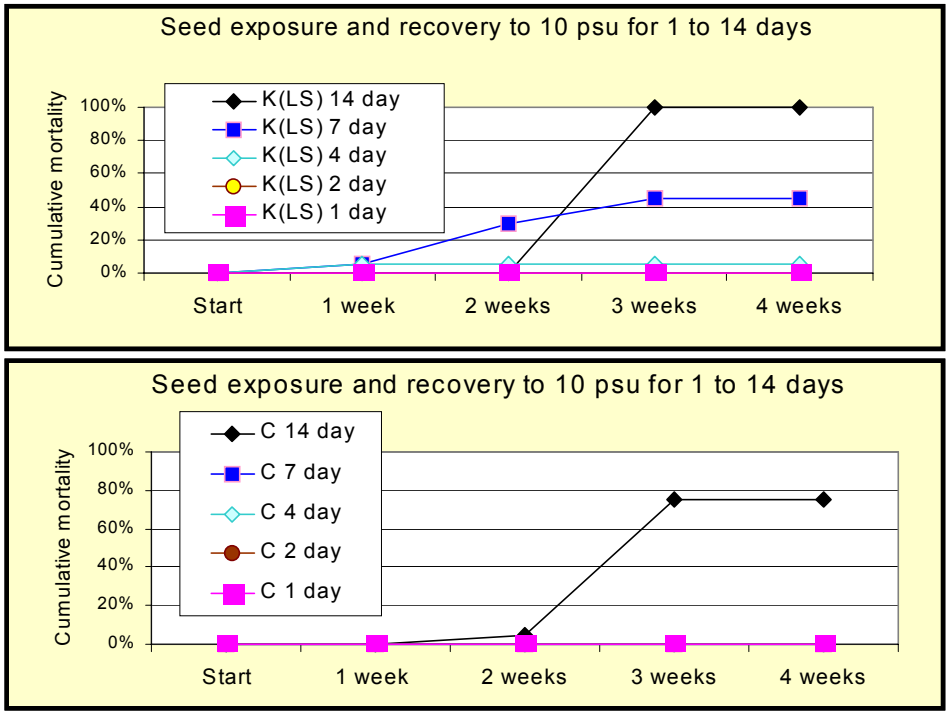


Figure 12. Response of two groups of seed clams to a salinity of 10 psu for variable times from 1 to 14 days.

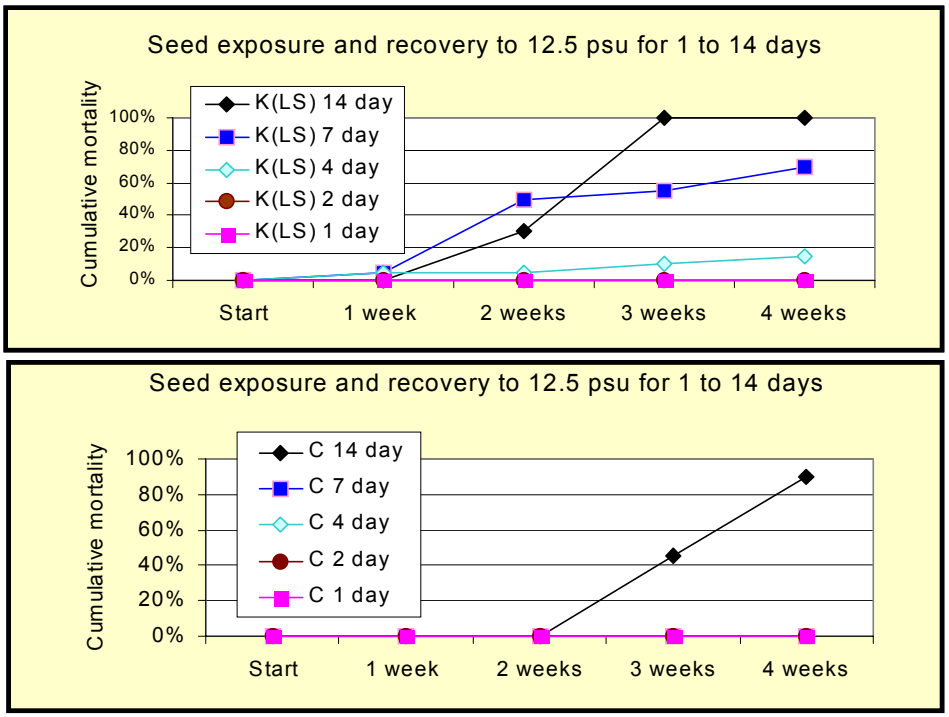


Figure 13. Response of two groups of seed clams to a salinity of 12.5 psu for variable times from 1 to 14 days.

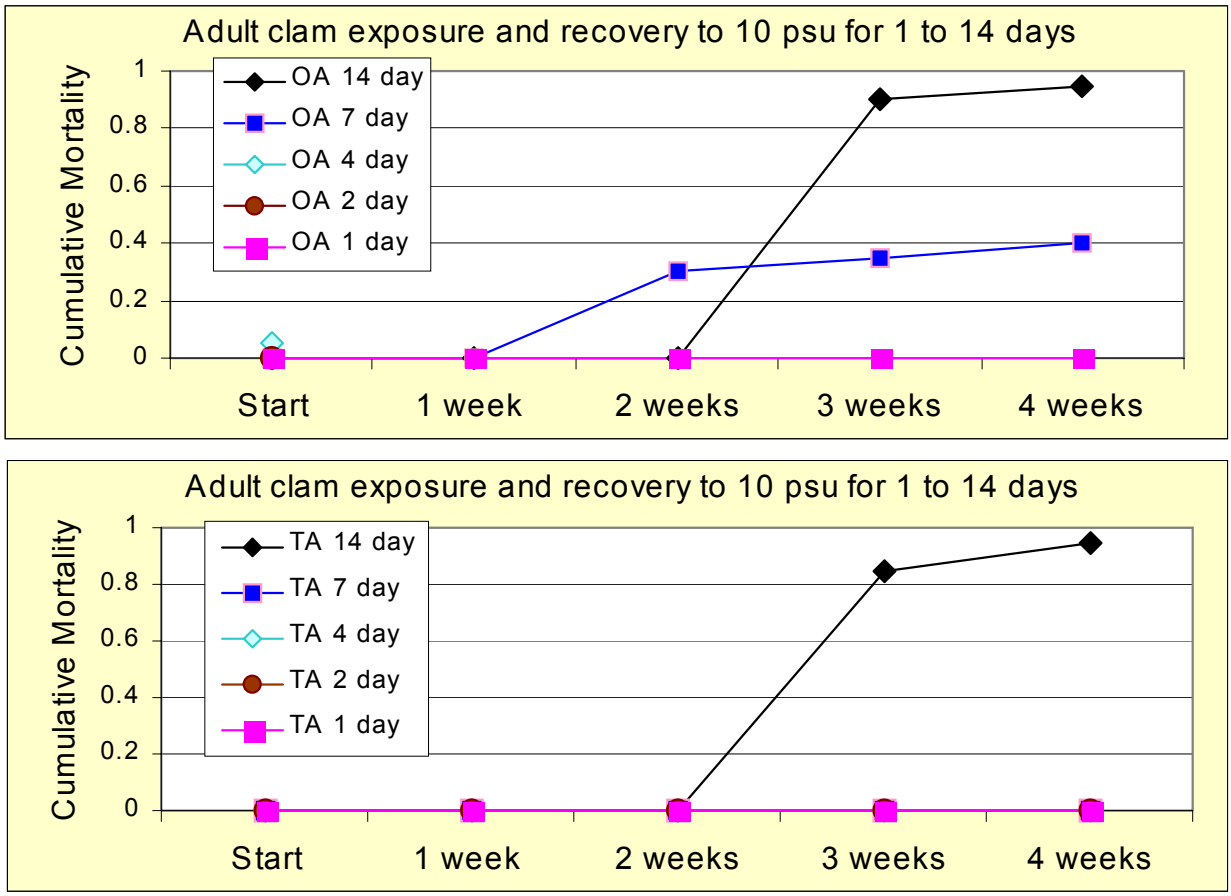


Figure 14. Response of two groups of adult clams to a salinity of 10 psu for variable times from 1 to 14 days.

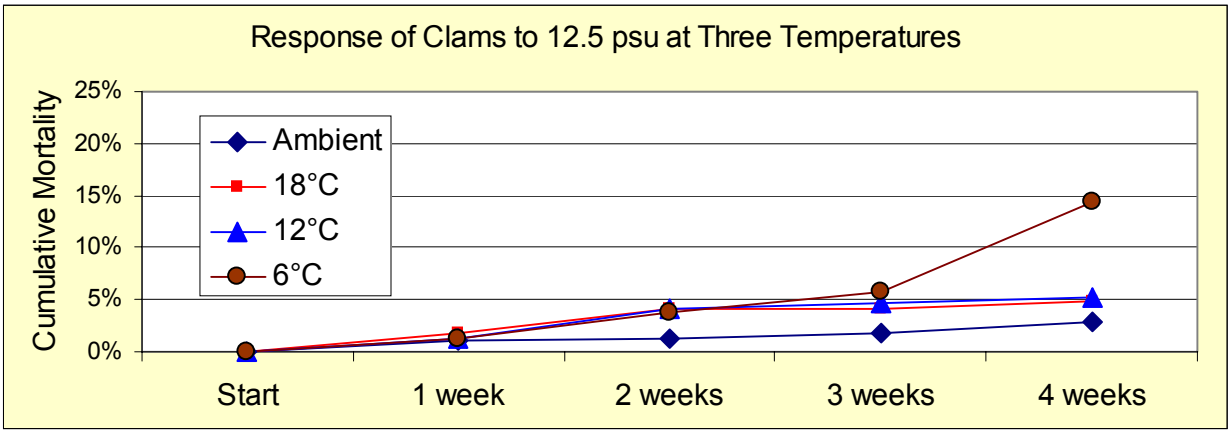


Figure 15. Mortality response of three groups of clams (date combined in graph) to the marginal salinity of 12.5 psu at three temperatures.

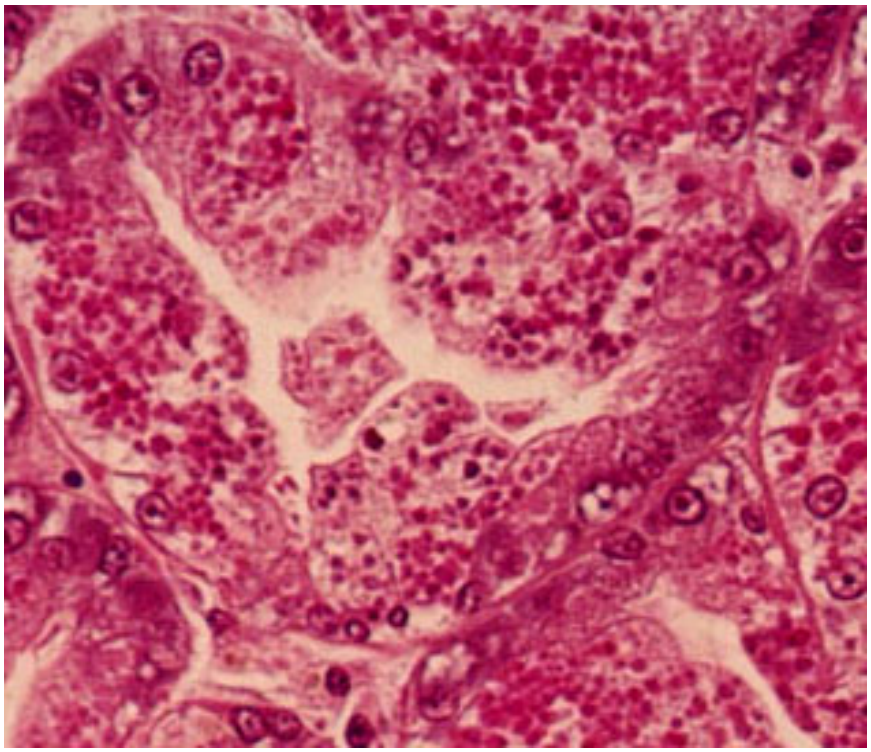


Figure 16. Normal digestive gland tubule from seed clam.

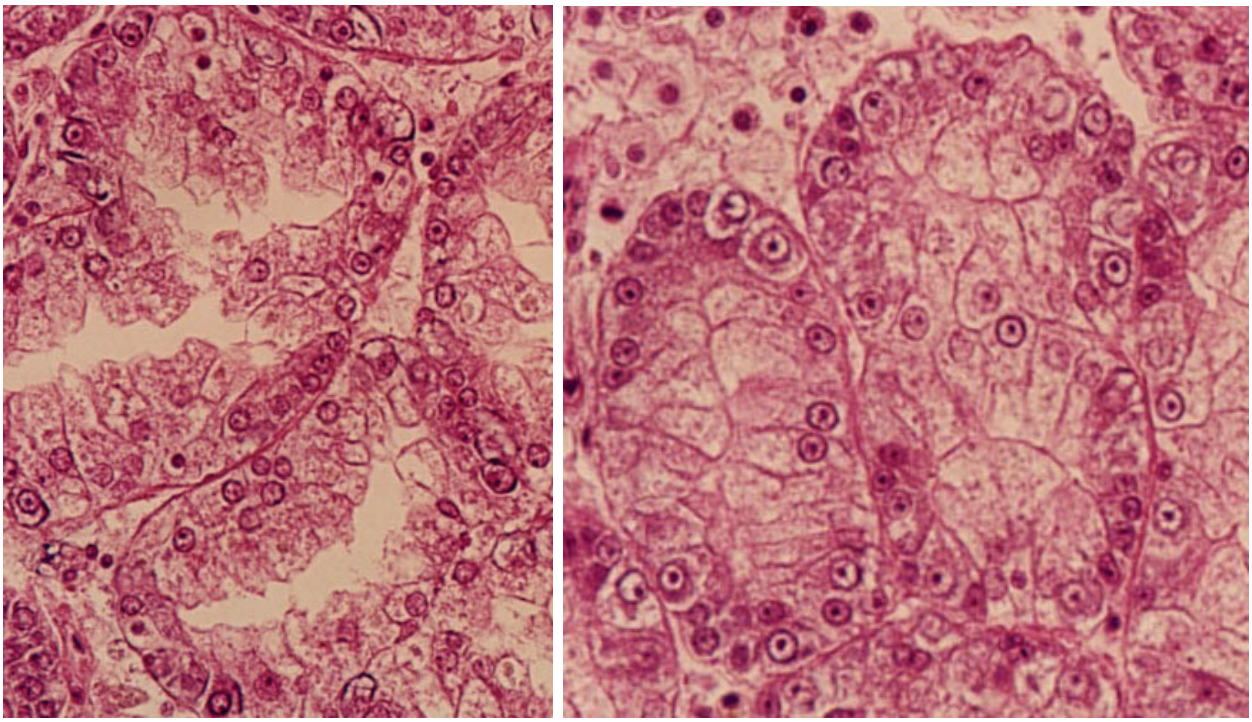


Figure 17. Degranulation of digestive gland cells (left panel) and subsequent osmotic swelling (right panel) resulting from low salinity exposure.